Note

Contribution of isozymic analysis in differentiating *Macrozamia moorei* D.L.Jones and K.D.Hill from *M.johnsonii* F.Muell (Zamiaceae)

Macrozamia johnsonii D.L. Jones & K.D. Hill was recently segregated from *M. moorei* F.Muell. (Jones and Hill, 1992) based on morphological characters, in particular, differences in the seedlings and plant habit. Populations of the two species are also disjunct, being separated geographically by a distance of about 800 km with *M. moorei* found in the central highlands of Queensland around Carnarvon Gorge and Springsure and *M. johnsonii* west of Grafton in north-eastern NSW. While the two species are morphologically distinct they are nevertheless closely allied and may represent sister taxa. The opportunity is taken here to compare enzyme banding patterns of both species using isozyme analysis.

Starch gel electrophoresis was employed to determine the enzyme pattern differences between two populations of *M. moorei* (Mt Zamia Environmental Park, *Forster,* PIF 14081, n=14; Staircase Range, *Forster and Machin*, PIF 9766, n=14 and one population of *M. johnsonii* (2.5km west of Bobtail Road, *P.Machin* s.n. (AQ540376), n=14). Both species have a narrow geographic range. Samples were collected from population extremes of each species and the voucher specimens housed at the Queensland Herbarium (BRI). When an extract of leaflet was assayed, following the method of Wendel and Weeden (1989), different but consistently reproducible electrophoretic banding patterns were produced (Fig. 1) for four of the eleven anodal enzyme systems assayed namely: Diaphorase (DIA, E.C 1.6.4.3), Menadione Reductase (MR, E.C 1.6.9.92), Uridine Diphosphogluconic Pyrophosphatase (UDP, E.C 2.7.7.9) and Isocitrate Dehydrogenase (IDH, E.C 1.1.1.42).

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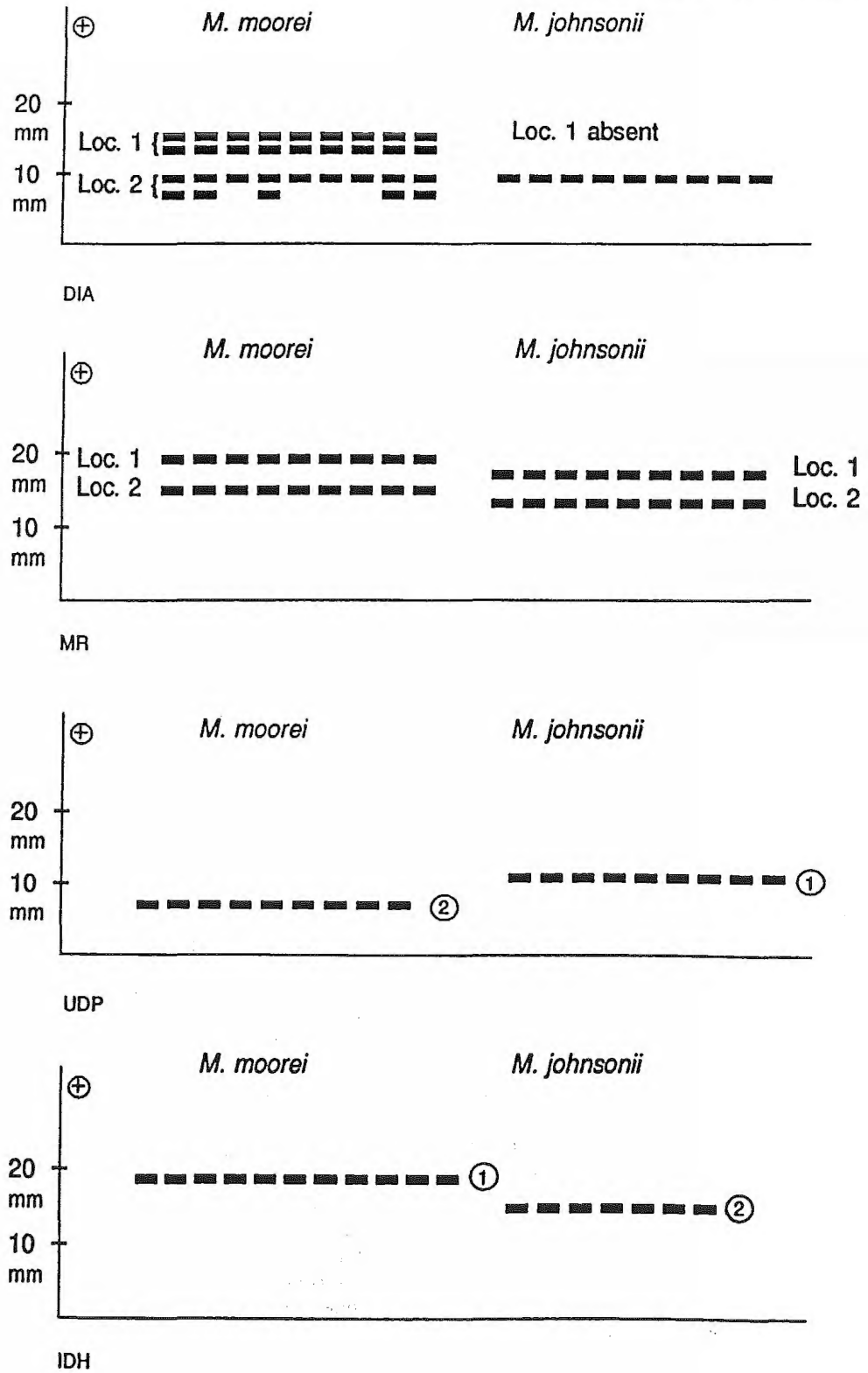
DIA: This monomeric enzyme exhibited a single band at locus one in all sampled plants of *M. moorei*, whereas the locus was absent in samples of *M. johnsonii*. At locus two *M. johnsonii* samples were monomorphic, but the locus was polymorphic in *M. moorei* with several of the plants examined being heterozygotes for the slower allelic variant.

MR: The two loci for this enzyme in samples of *M. moorei* and *M. johnsonii* revealed totally different positions and are presumed to be fixed for alternative alleles.

UDP: There were only two heterozygotes observed in samples of *M. moorei* with this monomeric enzyme (frequency of allele one =.07), the remaining samples being homozygotes at position two, whereas allele two was absent in samples of *M. johnsonii,* all being fixed at position one.

IDH: This dimeric enzyme produced only two types of homozygote zymograms. In all the samples of *M. moorei* examined, the locus was monomorphic at position one whereas in samples of *M. johnsonii* it was also monomorphic but at position two.

The absence of locus 1 in enzyme DIA for *M. johnsonii* and fixation of loci for alternate alleles in MR and IDH is deserving of further comment. There are three possibilities for these results to occur namely: (a) the same locus is present in both species but is fixed for alternative alleles at a different location (IDH, MR), (b) locus one for an enzyme DIA might be present but is fixed for allele overlapping locus 2 or a null allele or (c) there could have been two loci at IDH and MR in *M. moorei* and *M. johnsonii*, two loci at DIA in *M. johnsonii*, but one of them is presumed to have been lost during the course of evolution.


M. moorei
M. johnsonii
20 mm
10 mm
Loc. 1
Loc. 2
Loc. 1 absent
DIA
M. moorei
M. johnsonii
20 mm
10 mm
Loc. 1
Loc. 2
Loc. 1
Loc. 2
MR
M. moorei
M. johnsonii
20 mm
10 mm
1
2
UDP
M. moorei
M. johnsonii
20 mm
10 mm
1
2
IDH

In assessing allelic variation at the same loci, Nei's genetic distance (Nei, 1978) was calculated between the three populations sampled. The genetic distance between two congeneric species is generally considered to be 0.4 (Gottlieb, 1977) and the genetic distance between *M. moorei* and *M. johnsonii* was 0.48 (average of two populations of *M. moorei*) which supports the previous conclusion of Jones and Hill (1992) based on morphology, that these are distinct species. The low levels of genetic variability observance in *M. moorei* and virtually no variability observance in *M. johnsonii* may be due to the fact that the populations have been derived from a small number of plants and lack of gene flow from other populations has resulted in independent evolution of *M. moorei* and *M. johnsonii* with divergence caused mainly by random genetic drift. The low number of polymorphic loci and heterozygotes found in individuals may be attributed to any of the following factors: (a) the small and localised nature of the population, (b) a genetic "bottleneck structure" due to lack of gene flow from outside the population, (c) inbreeding between individuals within taxa, and (d) low sampling level.

The results obtained here provide supportive evidence for distinguishing *M. moorei* and *M. johnsonii* as distinct species.

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References

GOTTLIEB, L.D. 1977. Electrophoretic evidence and systematics. *Annals of Missouri Botanical Garden* 64: 161–180.

JONES, D.L. and HILL, K.D. 1992. *Macrozamia johnsonii*, a new species of *Macrozamia* section *Macrozamia* (Zamiaceae) from Northern New South Wales. *Telopea*. 5: 31–34.

NEI, M. 1978. Estimation of average heterozygosity and genetic distance from small numbers of individuals. *Genetics*. 9: 583–590.

WENDEL, J.F. and WEEDEN, N.F. (1989). Visualisation and interpretation of plant isozymes. In *Isozymes in Plant Biology*. Eds. D.E. Soltis and P.S. Soltis. pp. 5–45. Portland, Oregon: Discorides Press.

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